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Extending the Total Pore Blocking method to normal phase high performance liquid chromatography

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ABSTRACT

It has been demonstrated that it is possible to extend the Total Pore Blocking (TBP)-method for the determination of the external porosity (ε_e) of packed bed columns from reversed-phase (RP) chromatography to normal-phase (NP) chromatography conditions by switching the nature of the blocking agent and the interstitial void flushing liquid, i.e., by using a hydrophilic blocking agent (pure or buffered water at pH 3.0 or pH 7.0) and a hydrophobic flushing liquid (linear alkanes such as decane). Several parameters that might influence the accuracy of the method, such as the applicable range of flow rates and the meso-pore size of the particles have been investigated. The influence of several different parameters on the obtained external porosity value has been investigated. From a wide selection of possible t_0-markers, the class of linear alkanes has been shown to be the single possible one. This brings along the need to use refractive index detection to measure the signal of the linear alkane tracer (e.g., dodecane) in a stream of (another) linear alkane. The results of the newly established NP-TPB method have been compared to the values of the external porosity obtained by ISEC and proved to generate the same results, however with a much smaller read-out error (being only of the order of 0.1%).

1. Introduction

Several years ago, the Total Pore Blocking (TPB) method has been developed to determine the external porosity (ε_e) of reversed phase HPLC columns [1,2]. Knowing the value of the external porosity of a column is important since the ε_e-value is linked to the permeability and the flow resistance of a column through the Kozeny–Carman law. This law shows that a small difference in external porosity can lead to large differences in permeability and thus in flow resistance [3–6]. Since the permeability and the flow resistance are closely related to the performance of a column, knowing the value of ε_e of a column allows one to gain insight into the performance of the column [7,8].

The TPB technique has already been used in several studies [9–13]. The method consists of measuring the elution time of a non-retained small molecular weight tracer such as potassium iodide after having filled the micro- and meso-pores of the porous support with a hydrophobic liquid (decane) that is immiscible with the mobile phase employed during the elution time measurements (ammonium acetate buffer pH 3.0). The TPB method has been developed to provide an alternative to the inverse size exclusion chromatography (ISEC) that is commonly used to determine the external porosity ε_e [14–18]. Although it is rather slow, the TPB method has a few advantages over the ISEC method, such as the use of a small molecular weight marker (the large molecular weight polymers needed in ISEC cannot penetrate the smallest pockets of the interstitial void) and the unambiguous determination of the ε_e-value that can directly be read off from the measurement curves when steady-state column conditions have been achieved [1]. In ISEC, the elution volume of a series of polymers with different molecular weights is used to determine the external porosity of a column. The graphical analysis of ISEC is based on the determination of the intersection point of two straight lines, which is inclined to uncertainty since the drawing of these lines is based on a limited number of data points. This may lead to an error in ε_e of at least a few percent [19]. In the TPB-method, the external porosity is immediately calculated from the elution time of the small molecular weight marker, thus omitting the uncertainty on the determination of the intersection point of the two fitting lines in ISEC.

Up till now, ISEC still has an advantage over the TPB method because ISEC can be used in reversed phase as well as normal-phase conditions [20] while the TPB method is currently limited to reversed-phase conditions. However, considering the early days of chromatography, where normal-phase separations were conducted in liquid–liquid columns where a hydrophilic liquid was retained in porous particles, it should normally be possible to extend the TPB method to normal phase conditions. For example, Martin et al. [21] filled the pores of silica particles with water and

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used hexane as a mobile phase to conduct a form of partition chromatography to separate samples that are miscible in both liquids: the one used to fill the pores of the particles and the one used as mobile phase.

The present study therefore focuses on the extension of the TPB method to normal phase conditions by switching the nature of the blocking agent and the mobile phase as compared to reversed phase TPB. In normal-phase TPB (NP-TPB), the pores of the particles are filled with a polar liquid (such as water or ammonium acetate buffer) and the mobile phase consists of a hydrophobic liquid (such as decane). The influence of several parameters on the obtained ε_e-value has been investigated. After selecting the conditions that yield the most reliable determination of the external porosity, the results of the optimized NP-TPB method are compared to the results obtained by the ISEC method. To further investigate the validity of the NP-TPB method, and check whether the blocking agent can be fully flushed out of the interstitial void, an experiment has been performed on a column filled with nonporous silica particles to investigate whether the ε_e-value before and after having performed a NP-TPB experiment was the same.

2. Experimental

2.1. Chemicals and columns

Benzene (MW = 78.11 g/mol), methylbenzene (MW = 92.14 g/mol), ethylbenzene (MW = 106.17 g/mol), propylbenzene (MW = 120.19 g/mol), dodecylbenzene (MW = 246.43 g/mol), cyclohexane (MW = 84.16 g/mol), methylcyclohexane (MW = 98.19 g/mol), ethylcyclohexane (MW = 112.21 g/mol), heptane (MW = 100.21 g/mol), octane (MW = 114.23 g/mol), nonane (MW = 128.20 g/mol), undecane (MW = 156.31 g/mol), dodecane (MW = 170.34 g/mol) and the polystyrene standards with 12 different molecular weights ranging from 500 Da to 2,000,000 Da were purchased from Sigma–Aldrich (Steinheim, Germany). HPLC-grade isopropanol, decane (99+% pure), chloroform and tetrahydrofuran were purchased from Sigma–Aldrich (Steinheim, Germany). HPLC grade water was prepared in house using a Milli-Q Purification System (Millipore, Billerica, MA, USA). The Zorbax Rx-SIL column (4.6 mm × 150 mm, 80 Å pore size) filled with 5 μm particles was purchased from Agilent Technologies (Diegem, Belgium). The Sunfire Prep Silica column (4.6 mm × 150 mm, 94 Å pore size) filled with 5 μm particles was purchased from Waters (Zellik, Belgium). The column (2.1 mm × 100 mm) filled with nonporous 6.55 μm silica particles was provided by Thermo Fischer Scientific (Runcorn, UK).

2.2. Buffer

To conduct the NP-TPB experiments, a hydrophilic buffer was prepared that consisted of 10 mM ammonium acetate (Sigma–Aldrich, Steinheim, Germany) dissolved in Milli-Q water. To create a pH = 3-buffer, the pH was adjusted by adding acetic acid (Panreac, Barcelona, Spain).

2.3. Apparatus

Chromatographic data were acquired with an HPLC Agilent 1200 system (Agilent Technologies, Waldbronn, Germany) which can withstand pressures up to 600 bar. This instrument includes an auto-sampler with a 2 μl loop, a diode array detector with a 2 μl flow cell, and a column oven set at 30 °C. Data acquisition, data handling, and instrument control were performed using Chemstation (Agilent Technologies). Absorbance was measured using a diode array detector with a wavelength set at 210 nm, using a sampling rate of 40 Hz. The refractive index was measured by using an Agilent Technologies G1362A refractive index detector at a standard response time of 4 s. The optical unit was kept at a constant temperature of 35 °C and the detection was set at a positive polarity. Stainless steel tubing with an internal diameter of 120 μm and a length of 10.5 cm was used as connection pieces.

2.4. ISEC measurements

A standard ISEC protocol was used [19]. A series of polystyrene standards with known molecular mass was injected onto the column at a concentration of 0.1 mg/ml in tetrahydrofuran. The injection volume was 5 μl for each individual polystyrene standard. Flow rates of 0.2 ml/min, 0.4 ml/min and 0.6 ml/min were used. Methylbenzene was used as a marker to determine the total retention volume of the column. Each injection was performed in triplicate and the values shown in the graphs are the average of the three data points. All the retention volumes have been corrected for the extra-column band broadening of the system. The plot of the logarithm of the molecular mass of the polystyrene standards as a function of its retention volume was constructed by using the molecular mass corresponding to the peak maximum as specified by the supplier.

2.5. Pore blocking procedure

It is obvious that the first step of the TPB-procedure for reversed phase (i.e., rinsing the column with a solvent that is fully miscible with both the hydrophilic pore blocking liquid and the hydrophobic flushing liquid) can be preserved in the NP-TPB-procedure. The column is thus first rinsed with isopropanol at a flow rate of 1.000 ml/min for 60 min. In the next step the column is filled with a polar liquid (the blocking agent) that replaces the isopropanol in the micro- and meso-pores of the particles. In the present study, two blocking agents were used: water and ammonium acetate buffer (pH 3.0 and pH 7.0). The column was filled with blocking agent at a flow rate of 1.000 ml/min for 180 min. Finally, the blocking agent is flushed out of the interstitial space of the bed using a hydrophobic liquid which is immiscible with the hydrophilic blocking agent. The hydrophobic liquid used in the present study was decane. The samples were dissolved in decane to a final concentration of 1 mg/ml and were injected onto the column every 20 min (injection volume = 0.5 μl).

After flushing the column, the initially porous particles are expected to be transformed into fully blocked particles that are impermeable to hydrophobic solutes, and hence behave like nonporous particles. Injecting a t_0-marker into the hydrophobic mobile phase and recording its mean residence time leads to the volume of the interstitial space V_i by using the next equation:

$$V_i = F \times t_i \tag{1}$$

where F is the flow rate used for flushing the column (ml/min) and t_i is the elution time (min) of the injected marker.

The interstitial volume V_i needs to be corrected for the extra-column volume of the system V_{ext}. This volume was measured by replacing the column with a zero dead volume connection piece and was found to be 0.021 ml. The interstitial volume V_i also needs to be corrected for the volume of the frits (V_{frit}) that are present in the column and which correspond to a correction of 0.0023 ml [2]. The external porosity of the column can then be calculated by using the following equation:

$$\varepsilon_e = \frac{V_i - V_{ext} - V_{frit}}{V_{geom}} \tag{2}$$

with V_{geom} equal to $\pi r^2 L$ where r is the internal radius of the column and L is the length of the column. The Waters and Agilent columns